

## Population, morphological and antioxidant characteristics of species with resource potential of Zarafshan National Natural Park

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**Abstract:** The article presents the current state of populations, morphological, biochemical (antioxidants) and form diversity of *Hippophae rhamnoides* L., *Berberis integerrima* Bunge and *Crataegus pseudoheterophylla* subsp. *turkestanica* (Pojark.) K.I. Chr. in the territory of the Zarafshan National Nature Park (Republic of Uzbekistan). Against the background of increasing anthropogenic impact, a decrease in the number and density of individuals in the population, incompleteness of age spectra with the absence of pregenerative (seedlings, juvenile, immature), generative (young and old generative) and senile fractions, a shift in the sexual structure towards the predominance of males were established. A significant decrease in the diversity of *H. rhamnoides* forms is shown by combining the qualitative and quantitative morphological features of vegetative and reproductive organs, organoleptic properties and antioxidant activity. Two studied forms of *B. integerrima* were distinguished. All samples of juices from the fruits of *Hippophae rhamnoides*, *B. integerrima* and *C. pseudoheterophylla* subsp. *turkestanica* exhibits antioxidant properties and reduces the model substrate's oxidation rate by 2-5 times. The morphological forms of *B. integerrima* and *H. rhamnoides* differ in antioxidant activity. Among the forms of *H. rhamnoides*, samples 4, 5 and 6 have relatively high antioxidant properties. Form No. 2 of *B. integerrima* demonstrates the high antioxidant properties of the juice from the fruits. Among the studied species, the juice from the fruits

of *C. pseudoheterophylla* subsp. *turkestanica* has relatively high antioxidant properties.

**Keywords:** anthropogenic impact, form diversity, morphological features, pregenerative, population, senile fraction

### INTRODUCTION

The conservation of plant biodiversity represents one of the most urgent issues of our time. A substantial contribution to this is made by specially protected natural areas (SPNA), which serve as genetic reserves for the diversity of rare and resource species. [Methodology..., 2020; Barlybayeva, Ishmuratova, 2020; Kholbutayeva et al., 2020]. More than 300 species of vascular plants grow on the territory of the Zarafshan National Nature Park (ZNNP) [Kholbutayeva et al., 2020], including resource (food, medicinal, vitamin, oilseed, etc.) species. However, a change in the conservation status of SPNA towards easing the forms of protection, as a rule, leads to an increase in anthropogenic impact and, as a consequence, a decrease in biodiversity and the loss of unique rare forms. For over 30 years, *Hippophae rhamnoides* L. populations have been studied in the ZNNP, formerly known as Zarafshan Nature Reserve. The identification of morphological forms based on the following parameters: spinosity, shape, size and color of the fruit, content of oil, carotenoids, vitamins, etc. [Kabulova, Turdyeva, 2014]. Currently, studies are underway on the current state of populations [Ishmuratova, Kabulova, Marmazinskaya, 2023] and reserves of *H. rhamnoides* raw materials in the Zarafshan River valley [Erdonov et al., 2023]. Many fruit and berry plants are both rare species in various parts of their range and valuable sources of vitamins, biologically active substances and antioxidants. This significantly increases the need to organize their protection to preserve the genetic material of adapted populations that are promising for further breeding work. Among such species, we can highlight *H. rhamnoides*, *Berberis integerrima* Bunge and *Crataegus pseudoheterophylla* subsp. *turkestanica* (Pojark.) K. I. Chr., which are currently cultivated as crops, but their populations in different parts of their ranges require

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comprehensive study and conservation. The work aims to study the current state of morphological, antioxidant and form diversity of populations of some resource plant species in the territory of the ZNPP.

#### MATERIAL AND METHODS

*Description of the study area.* Population studies of resource species and selection of forms were carried out in 2023 on the territory of the ZNPP (Fig. 1), coordinates latitude 39°36'21"N longitude 67°11'21"E. The objects of the study were *Hippophae rhamnoides* L., *Berberis integerrima* Bunge and *Crataegus pseudoheterophylla* subsp. *turkestanica* (Pojark.) K. I. Chr. The Latin names of the plant species described in this work are given according to the World Flora Online.

On the territory of 3.5 hectares, 10 sample plots of 15 m<sup>2</sup> each were laid out. The age and sex structure of *H. rhamnoides* populations, the number and density of partial shoots in clones, and the degree and form of anthropogenic impact were determined [Methodology..., 2020].

The identification of *H. rhamnoides* forms was carried out based on 17 qualitative and quantitative morphological characteristics of vegetative (plant height (m), shape, density and type of crown, angle of departure of the main skeletal branches, thorniness, length of thorns (cm), leaf colour) and reproductive (degree of fruiting, number of fruits per node (pcs.), length of peduncle (mm), colour, shape, length and width (mm), nature of separation, weight of 100 fruits (g)) organs; organoleptic (taste) and biochemical (antioxidant activity) characteristics of fruits [Kabulova, Turdyeva, 2014]. Female plants in the middle-aged generative state were selected. *B. integerrima* forms were distinguished based on 9 qualitative and quantitative morphological characteristics of vegetative (number (pcs.) and length of shoots (m), length of thorns (cm) on a shoot) and reproductive (number of fruiting clusters on one shoot (pcs.), number of fruits in one cluster (pcs.), fruit colour, length and diameter of fruit (mm), weight of 100 fruits (g)) organs; and biochemical (antioxidant activity) characteristics of fruit juice.

The office processing of the research results was carried out in the laboratory of Reproductive Biology and Plant Cloning of the Institute of Nature and Man of the Ufa University of Science and Technology (UUNiT). The determination of the antioxidant activity of the fruits was carried out at the Department of Physical Chemistry and Chemical Ecology of the Institute of Chemistry and Protection in Emergencies of UUNiT.

Fruits of *H. rhamnoides*, *B. integerrima* and *C.*



**Figure 1.** Location of Zarafshan State National Park (Republic of Uzbekistan).

*Note:* The riverbed of the Zeravshan River is marked in yellow, and the border of the national park is marked in white.

*pseudoheterophylla* subsp. *turkestanica* was collected from plants in their natural habitats during the ripening period in October 2023 to determine their antioxidant activity. The antioxidant activity of the juice was studied using the example of a model reaction of initiated radical chain oxidation of ethylbenzene (EB), which is a good model substrate [Emanuel, Gal, 1984]. The classic initiator, azodiisobutyronitrile (AIBN), was used as an initiator of the oxidation process. The amount of absorbed oxygen was measured using a highly sensitive universal differential setup that allows measuring oxygen absorption rates within 10<sup>-6</sup>-10<sup>-7</sup> mol/L\*sec [Garifullina et al., 2022]. The experiments were carried out at 348 K. The juice from the fruits was mechanically isolated and passed through a filter to remove the solid phase. Various volumes of freshly squeezed fruit juices were added to the oxidized substrate in the reactor of the setup.

#### RESULTS

Previously, we studied the current state of *H. rhamnoides* populations [Ishmuratova, Kabulova, Marmazinskaya, 2023] in the territory of the ZNPP. It was shown that the age spectra of clones are incomplete, with the absence of pregenerative (sprouts, juvenile, immature), generative (young and old generative) and senile fractions. By now, the number of females in these same populations has significantly decreased (to 16-34 pcs.), and the proportion of males has increased (to 11-89 pcs.). The change in the

sex structure of *H. rhamnoides* populations towards the predominance of males occurs against the background of the intense anthropogenic impact associated with the use of unacceptable methods of harvesting fruits: cutting and breaking off fruiting branches. Such intensive use of natural *H. rhamnoides* thickets is associated with a change in the status.

ZNPP, where the main thickets of *H. rhamnoides* are concentrated, is located in the southeastern part of the Samarkand region along the Zarafshan river (Fig. 1). In 2018, based on the Resolution of the Cabinet of Ministers of the Republic of Uzbekistan "On the organization of the activities of the Zarafshan National Nature Park", the protected area changed its status from the category of a

reserve to the category of a national nature park. In this regard, there is currently an increase in anthropogenic impact on unique tugai ecosystems in general, and on communities with *H. rhamnoides*.

Earlier [Kabulova, Turdyeva, 2014] on the territory of the ZNPP, which is the centre of distribution and form diversity of natural *H. rhamnoides* thickets in the republic, 17 morphological forms of *H. rhamnoides* were identified. To date, only 6 forms of *H. rhamnoides* have been identified based on a combination of qualitative and quantitative morphological features of vegetative and reproductive organs, and organoleptic properties (Tables 1, 2). Important economically valuable qualities for fruit and berry plants include the size of the crown,

**Table 1.** Morphological characteristics of vegetative organs of *Hippophae rhamnoides* in the Zarafshan National Nature Park (Republic of Uzbekistan).

Sample number	Plant height, m	Crown		The angle of divergence of skeletal branches	Plant thorniness	Length of thorns on fruit-bearing shoot, cm	Leaf colouring
		shape	density				
1	2, 7	spreading	very thick	35°	-	-	greenish-silver
2	3,5	conical	average	45°	weak	4,0-8,0	greenish-silver
3	4,5	oval	average	40°	weak	1,5-3,0	greenish-silver
4	4,3	conical	average	45°	average	3,5-4,0	greenish-silver
5	4,2	oval	average	45°	strong	2,0-4,0	greenish-silver
6	4,7	conical	thick	45°	weak	2,0-3,0	green

**Table 2.** Morphological characteristics and organoleptic properties of the reproductive organs of *Hippophae rhamnoides* in the Zarafshan National Nature Park (Republic of Uzbekistan).

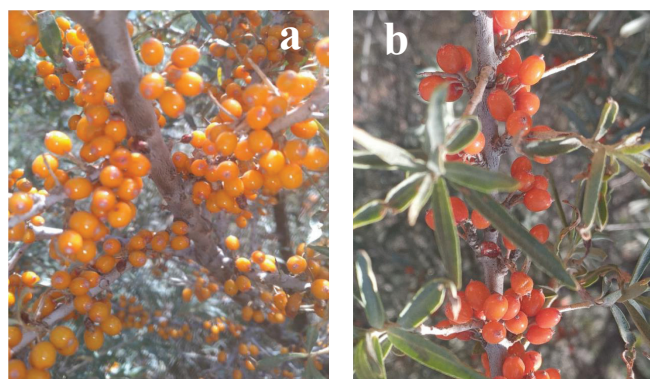
Sample number	Number of fruits in one fruit bud, pcs.	Length of stalk, mm	Fetus				Weight of 100 fruits, g	Taste	
			colouring	form	length, mm	width, mm			nature of separation
1	4-6	0,2-0,3	red	oval	0,5-0,6	0,45-0,5	wet	12,9	sour
2	4-6	0,3-0,4	yellow	oval	0,6-0,7	0,5-0,6	dry	11,7	sweet and sour
3	4-7	0,3-0,5	yellow-orange	cylindrical	0,6-0,8	0,4-0,5	wet	11,1	sour
4	6-8	0,2-0,4	yellow	oval	0,5-0,7	0,5-0,6	wet	10,2	sweet and sour with bitterness
5	3-6	0,3-0,5	orange	oval	0,45-0,5	0,4-0,5	wet	8,2	sour
6	4-6	0,4-0,5	reddish orange	oval	0,7-0,75	0,5-0,6	dry	13,7	sour



thorniness, number, size, and weight of fruits, the content of biologically active substances and their properties, and organoleptic properties. A description of *H. rhamnoides* samples based on qualitative and quantitative morphological features of vegetative organs is presented in Table 1. The resource value of the analyzed thickets is berry, and the degree of fruiting of the plants is medium and high. The height of fruit-bearing *H. rhamnoides* plants in the population varies from 2.7 to 4.7 m. At the same time, tall (above 4 m) plants and medium-sized (2.7-3.5 m) plants can be distinguished. The crown shape varies from spreading to oval and conical, and the crown density from medium to dense and very dense. Of particular interest for breeding work is the degree of thorniness: the population contains plant forms from thornless (sample 1) to strongly thorny (sample 5).

The morphological characteristics and organoleptic properties of the reproductive organs of *H. rhamnoides* are presented in Table 2. The number of fruits in one node can vary from 4 to 8 pcs. A relatively high number of fruits in one node is noted in sample 4. The colour of berries varies from yellow to red (Fig. 2), and the shape is oval and cylindrical. Taste qualities range from sour to sweet and sour and sweet and sour with bitterness. Sample 5 is characterized by relatively small fruits, and sample 6 by relatively large ones. Weight of 100 pcs. fruits is relatively high in samples 1 and 6 (12.9 and 13.7 g, respectively) and low (8.2 g) in sample 5.

Two morphological forms of *B. integerrima* (Table 3) were identified, differing in the morphological characteristics of the vegetative and reproductive organs, and the color of the fruits (Fig. 3). Form *B. integerrima* No. 1 is characterized by red-burgundy and relatively small fruits, form No. 2 - by black-violet, relatively large and heavy (weight of 100 pcs.) fruits.



**Figure 2.** The colour of the fruits of *Hippophae rhamnoides* is yellow (a) (sample 2), red (b) (sample 1).



**Figure 3.** Morphological forms of *Berberis integerrima* No. 1 (a), No. 2 (b).

*Hippophae rhamnoides*, *B. integerrima* and *C. pseudoheterophylla* subsp. *turkestanica* are valuable food and medicinal plants containing a wide range of biologically active substances, including vitamins and antioxidants. The antioxidant activity of the fruit juices of the studied species, estimated by the oxidation rate of ethylenebenzene, is presented in Table 4. All samples of fruit juices of the studied species demonstrate antioxidant properties, reducing the oxidation rate of the model substrate by 2-5 times. Juice from the fruits of *C.*

**Table 3.** Morphological characteristics of various forms of *Berberis integerrima* in the Zarafshan National Nature Park (Republic of Uzbekistan).

Morphological feature (min-max)	Form 1	Form 2
Number of shoots on a bush, pcs.	22-25	15-35
Length of shoots, m	1,1-1,5	1,5-1,8
Length of thorns on shoots, cm	1,2-4,5	0,7-4,9
Number of fruiting clusters on one shoot, pcs.	20-31	15-35
Number of fruits in one cluster, pcs.	20-31	16-33
Fruit color	red-burgundy	black-violet
Fruit length, mm	7,0-7,4	7,7-8,5
Fruit diameter, mm	4,8-5,3	6,2-6,6
Weight of 100 fruits, g	4,5-13,0	22,0-26,0

**Table 4.** Dependence of the oxidation rate of ethylenebenzene upon addition of juice from the fruits of *Hippophae rhamnoides*, *Berberis integerrima* and *Crataegus pseudoheterophylla* subsp. *turkestanica*.

Type sample number, shape number	$v \cdot 10^6 \text{ mol/l} \cdot \text{s} \text{ (1)}$	
	Volume fraction of juice, ml(3)	
	$\varphi=0,$ 125	$\varphi=0,2$ 50
EB+AIBN <sup>(2)</sup>	8,00	8,00
<i>H.rhamnoides</i> sample 1	5,06	3,26
<i>H.rhamnoides</i> sample 2	5,69	4,03
<i>H.rhamnoides</i> sample 3	5,14	3,52
<i>H.rhamnoides</i> sample 4	4,38	2,16
<i>H.rhamnoides</i> sample 5	4,95	2,99
<i>H.rhamnoides</i> sample 6	4,79	2,55
<i>B. integerrima</i> form 1	3,91	2,07
<i>B. integerrima</i> form 2	3,33	1,90
<i>C. pseudoheterophylla</i> subsp. <i>turkestanica</i>	2,58	1,52

**Note:** Ethylbenzene oxidation rate without additives and with juice additives. 2. Model reaction system consisting of ethylbenzene (EB) and an initiator – azodiisobutyronitrile (AIBN). 3. The volume fraction of juice in the reaction is equal to the ratio of the volume of juice in ml to the volume of the oxidized system (dimensionless value)

*pseudoheterophylla* subsp. *turkestanica* has relatively high antioxidant properties. When it is added in a volume fraction of 0.25%, the oxidation of ethylbenzene slows down by 5.3 times. Juice from the fruits of *B. integerrima* turned out to be slightly weaker. The antioxidant activity of the juice of the fruits of *B. integerrima* form 2 is higher than that of form 1.

Among the forms of *H. rhamnoides*, samples 4, 5 and 6 have relatively high antioxidant properties. The dependence of the effect of reducing the rate of EB oxidation on the volume fraction in the reaction mixture was established: the higher the volume fraction, the higher the effect of inhibiting the oxidation reaction. Thus, sample 4 of *H. rhamnoides* reduces the rate of the oxidation process from  $8.00 \cdot 10^{-6} \text{ mol/L}$  to  $2.164 \cdot 10^{-6} \text{ mol/L}$ , i.e. almost 4 times.

The authors of the article declare no conflict of interest.

## CONCLUSION

Conservation of biodiversity of resource plant species is a pressing issue of our time. An important role in this regard is played by protected areas, where populations of many native plant species are concentrated. Changing the status of a protected area from a reserve to a national or natural park leads to a decrease in the strictness of protection and an increase in the anthropogenic load on the communities and populations of protected objects.

As a result, there is an increase in the exploitation of ecosystems and a decrease in the level of biodiversity.

To date, in the territory of the Zarafshan State Reserve, against the background of intensified anthropogenic impact, a decrease in intraspecific and population (age and sex structure) biodiversity of *H. rhamnoides* is observed. The surviving forms of *H. rhamnoides* are individual in terms of the combination of qualitative and quantitative morphological features of vegetative and reproductive organs, organoleptic properties and antioxidant activity.

All samples of juices from the fruits of *Hippophae rhamnoides*, *B. integerrima* and *C. pseudoheterophylla* subsp. *turkestanica* demonstrate antioxidant properties and reduce the oxidation rate of the model substrate by 2-5 times. The two studied forms of *B. integerrima* also differ in antioxidant activity. Among the forms of *H. rhamnoides*, samples 4, 5 and 6 have relatively high antioxidant properties. Among the studied species, the juice from the fruits of *C. pseudoheterophylla* subsp. *turkestanica* has relatively high antioxidant properties.

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### **Zərəfşan Milli Təbiət Parkının resurs potensialına malik növlərinin populyasiyası, morfoloji və antioksidant xüsusiyyətləri**

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Məqalədə Zərəfşan Milli Təbiət Parkının ərazisində (Özbəkistan Respublikası) *Hippophae rhamnoides* L., *Berberis integerrima* Bunge və *Crataegus pseudoheterophylla* subsp. *turkestanica* (Pojark.) K. I. Chr. növlərinin populyasiyalarının mövcud vəziyyəti, morfoloji, biokimyəvi (antioksidant) forma müxtəlifliyi təqdim olunur. Artan antropogen təsir fonunda populyasiyada fərdlərin sayının və sıxlığının azalması, yaş spektrlərinin natamamlığı ilə pregenerativ (cücərti, yuvenil, immatur), generativ (gənc və yaşlı generativ) və senil fraksiyalarında cinsi quruluşun erkək fərdlərin dominantlığına doğru dəyişməsi müəyyən edilmişdir. *H. rhamnoides* formalarının müxtəlifliyində əhəmiyyətli dərəcədə azalması, vegetativ və reproduktiv orqanların keyfiyyət və kəmiyyət morfoloji xüsusiyyətlərinin, orqanoleptik və antioksidant aktivliyin birləşməsinə əsaslanaraq göstərilmişdir. *B. integerrima* növünün iki morfoloji forması müəyyən edilmişdir. *Hippophae rhamnoides*, *B. integerrima* və *C. pseudoheterophylla* subsp. *turkestanica* meyvələrindən alınan bütün şirə nümunələri antioksidant xüsusiyyətlər nümayiş etdirir və model substratın oksidləşmə sürətini 2-5 dəfə azaldır. *B. integerrima* və *H. rhamnoides* antioksidant fəaliyyəti ilə fərqlənir. *H. rhamnoides* formaları arasında 4, 5 və 6-cı nümunələr nisbətən yüksək antioksidant xüsusiyyətlərə malikdir. *B. integerrima* növünün 2 nömrəli formasının meyvə şirəsi yüksək antioksidant xüsusiyyətlər nümayiş etdirir. Tədqiq olunan növlər arasında *C. pseudoheterophylla* subsp. *turkestanica* meyvələrindən alınan şirə nisbətən yüksək antioksidant xüsusiyyətlərə malikdir.

**Açar sözlər:** antropogen təsir, forma müxtəlifliyi, morfoloji xüsusiyyətlər, pregenerativ, populyasiya, senil fraksiya

### **Популяционная, морфологическая и антиоксидантная характеристика видов с ресурсным потенциалом Зарафшанского национального природного парка**

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В статье обсуждается современное состояние популяций, морфологическое, биохимическое (антиоксиданты) формовое разнообразие *Hippophae rhamnoides* L., *Berberis integerrima* Bunge и *Crataegus pseudoheterophylla* subsp. *turkestanica* (Pojark.) K.I. Chr. встречающихся на территории Зарафшанского национального природного парка (Республика Узбекистан). На фоне усиления антропогенного воздействия установлено снижение численности и плотности особей в этих популяциях, неполночленность возрастных спектров с отсутствием прегенеративных (проростки, ювенильные, имматурные), генеративных (молодые и старые генеративные) и сенильных фракций, смещение половой структуры в сторону

преобладания мужских особей. Показано существенное сокращение разнообразия форм *H. rhamnoides* по сочетанию учитываемых качественных и количественных морфологических признаков вегетативных и репродуктивных органов, органолептическим свойствам и антиоксидантной активности. Выделены две морфологические формы *B. integerrima*. Все образцы соков из плодов *Hippophae rhamnoides*, *B. integerrima* и *C. pseudoheterophylla* subsp. *turkestanica* проявляют антиокислительные свойства и снижают скорость окисления модельного субстрата в 2-5 раз. Морфологические формы *B. integerrima* и *H. rhamnoides* различаются по показателям антиоксидантной активности. Среди форм *H. rhamnoides* относительно высокими антиокислительными свойствами обладают образцы 4, 5 и 6. Форма № 2 *B. integerrima* демонстрирует высокие антиокислительные свойства сока из плодов. Относительно высокими антиоксидантными свойствами, среди исследованных видов, обладает сок из плодов *C. pseudoheterophylla* subsp. *turkestanica*.

**Ключевые слова:** антропогенное воздействие, формовое разнообразие, морфологические особенности, прегенеративная, популяция, сенильная фракция